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THE EFFECT OF COLCEMID ON THE HEAT SURVIVAL OF MITOTIC V79 CHINESE HAMSTER CELLS

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Abstract

V79 Chinese hamster cells were collected by colcemid addition to study the effect of heat on mitosis. When they were heated at 42°C and 45°C in the presence of 0.06 μg/mL colcemid, cell survival increased over the control samples, which were heated in ordinary medium. Scanning electron microscopy showed that cells heated to 45°C in the presence or absence of colcemid had fewer microvilli on the surface, but they did not have increased bleb formation. Transmission electron microscopy showed that the chromatin was diffuse in the heated cells and the kinetochores were indistinct. The mitochondria in the heated cells were also swollen and contained visible particles.

Key Words: Colcemid, Hyperthermia, V79 cells, scanning electron microscope, electron microscope, survival, mitosis.

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Introduction

The effects of hyperthermia on normal and tumor cells have been studied and reviewed extensively (Dewey, 1989; Raaphorst and Szekely, 1988; Dethlefsen and Dewey, 1982; Love et al., 1970). A number of these reports have shown that response to hyperthermia is dependent upon position in the cell cycle: cells are generally most sensitive at mid S-phase and mitosis. In order to study cellular sensitivity in mitosis, V79 cells were treated with colcemid, which disrupts the microtubules and holds cells in mitosis at 37°C. During the course of the experiments, some cells were heated in the presence of colcemid. It was noted that those heated in medium with colcemid survived better than those heated without it. In order to study this further by electron microscopy and clonal survival, mitotic cells were collected and heated in the presence and absence of colcemid to observe their survival and morphology.

Materials and Methods

The cells used were Chinese hamster lung fibroblasts derived from the V79-S171 cell line and designated V79-S171-W1. They were set up 40 to 48 h prior to the start of the procedure in 75 cm^2 tissue culture flasks at a density of about 2.5×10^5 cells/flask in 25 mL of medium. Cells in exponential phase were maintained in a 50:50 mixture of Dulbecco's Modified Eagle medium and Ham's F12 medium containing 10% heat inactivated fetal calf serum and 25 μg/mL gentamicin sulfate. Further detail of cell culturing and incubation are described elsewhere (Borsa et al., 1984).

The exponentially growing population was synchronized in mitosis by adding 0.06 μg/mL colcemid in a 1:1 mixture of conditioned and fresh medium to the culture for 3 h. After the 3-h incubation, the flasks were shaken to remove the rounded, loosely attached mitotic cells. The mitotic index was greater than 90% in all experiments. The sample was resuspended in 10 mL of a 1:1 mixture of conditioned and fresh medium, divided into two centrifuge tubes and one half of the sample was inoculated with 0.06 μg/mL colcemid. The centrifuge tubes were sealed with wax and heated in water baths with a

Table 1. Survival of mitotic V79 cells after heat treatment at 42°C.

heating time	Plating Efficiency (PE)				Relative PE
	without colcemid		with colcemid		$\frac{PE_{42°C+colcemid}}{PE_{42°C}} \div \frac{PE_{37°C+colcemid}}{PE_{37°C}}$
(h)	37°C	42°C	37°C	42°C	
1	46.8	29.1	38.6	37.6	1.57
1	61.0	11.7	ND	14.6	1.25+
1	28.9	20.4	25.6	27.2	1.51
1	24.3	13.0	11.7	25.4	4.06
5	54.0	8.3	46.2	10.5	1.48
6	14.2	1.7	20.1	3.6	1.50

ND = not done

+ based on $PE_{37°C+colcemid} = PE_{37°C}$

Table 2. Survival of mitotic V79 cells after heat treatment at 45°C.

heating time	Plating Efficiency (PE)				Relative PE
	without colcemid		with colcemid		$\frac{PE_{45°C+colcemid}}{PE_{45°C}} \div \frac{PE_{37°C+colcemid}}{PE_{37°C}}$
(h)	37°C	45°C	37°C	45°C	
5	41.1	24.2	41.8	33.5	1.36
10	46.4	17.8	45.7	20.1	1.15
10	43.2	8.6	34.8	9.2	1.33
15	ND	1.6	ND	3.9	ND
20	ND	0.1	26.2	0.2	1.73
21.5	49.5	0.2	39.6	0.2	1.01
30	40.8	0.1	38.9	0.1	1.09

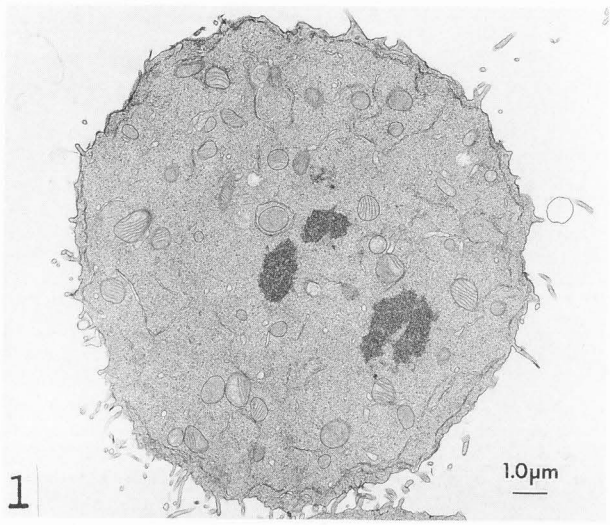
ND = not done

+ based on $PE_{37°C+colcemid} = PE_{37°C}$

temperature control of +/-0.02°C before being plated for survival measurements by clonal survival or fixed for electron microscopy. The whole mitotic collection process was carried out in a 37°C warm-room.

For scanning electron microscopy, V79 Chinese hamster cells were grown on Lux Thermanox coverslips in order to observe both mitotic and interphase cells after the heat treatment. For transmission electron microscopy, the cells were pelleted after heat treatment. Both scanning and transmission electron microscopy samples were prepared by standard methods (Szekely et al., 1989).

The cells were plated into four replicate 25-cm² flasks containing 5 mL of F-12 fresh medium. Seven to ten days later, macroscopic colonies, attached to the flask, were scored. The survival data are corrected for multiplicity by standard procedures (Elkind and Whitmore, 1967).



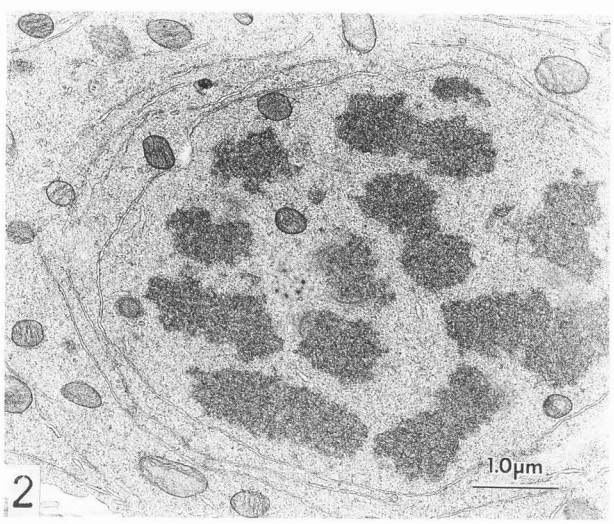
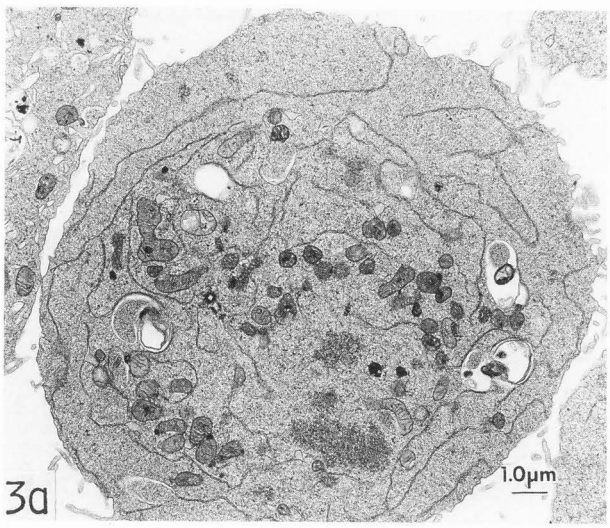


Fig. 1. Micrograph of a V79 Chinese hamster cell that was collected in mitosis by mitotic shake after colcemid treatment and resuspended in fresh media without colcemid for 10 min at 37°C.

Fig. 2. The central area of a mitotic cell that was held at 37°C for 30 min in the presence of colcemid.



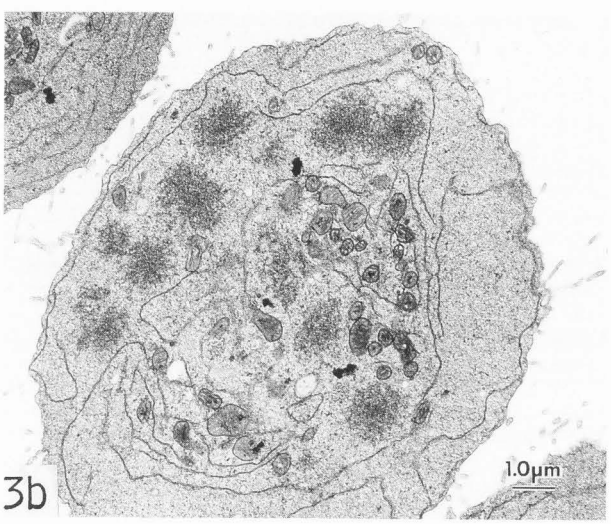


Fig. 3. V79 cells that were collected in mitosis by mitotic shake after colcemid treatment and resuspended in medium for a 20-min incubation at 45°C (a) without colcemid and (b) with colcemid.

Results

The survival of mitotic V79 cells was measured after heat treatment in the presence or absence of colcemid. The data are summarized in Tables 1 and 2. The plating efficiencies (PE) at 37°C are probably low and variable because of the manipulation required before the cells were plated. The paired observations of PE with and without colcemid present during the 37°C incubation were generally lower for the colcemid-treated cells. After the heat exposure of 42°C or 45°C, the level of survival dropped, as expected; however, the mitotic cells heated in the presence of colcemid showed a higher survival level in all experiments except one. The paired observations were tested using a distribution-free signed rank Wilcoxon test (Hollander and Wolfe, 1973). At 37°C, cell survival without colcemid was not significantly higher than that with colcemid at the $p = 0.05$ level in a one-tailed test. In the cells heated to 42°C and 45°C, however, the cell survival was significantly lower ($p = 0.05$) in the cells without colcemid compared to those heated with it. The effect was accentuated if the plating efficiencies were expressed as a relative survival value,

$$\frac{(PE_{\text{hyperthermia+colcemid}} / PE_{\text{hyperthermia}})}{(PE_{37°C+\text{colcemid}} / PE_{37°C})}$$

which was calculated for each heating condition. The relative survival value increased in the presence of colcemid. The values were significantly greater than 1.0 at the $p = 0.05$ level using the signed rank statistic.

Transmission electron microscopy was used to observe the morphology of the treated cells before plating. Typical control cells, which were incubated at 37°C, are shown in Figures 1 and 2. The cell incubated in the presence of colcemid, shown in Figure 2, has its endoplasmic reticulum moved toward the center of the cell. This was a common feature of many cells incubated in the presence of colcemid at various temperatures. The mitochondria in both colcemid-treated and untreated cells are well formed and the kinetochores are visible in some sections. As we have reported earlier (Szekely et al., 1983), colcemid-treated cells frequently have bands of microfilament cables around their periphery.

In the 45°C heated cells (Figures 3a and 3b), the chromosomes appear diffuse and the kinetochores are not visible. Mitochondria are frequently disrupted and without visible cristae. Mitochondrial particles are also visible. There does not appear to be a noticeable difference in morphology between those incubated in the presence or absence of colcemid; although once more, the mitochondria and other cytoplasmic organelles are gathered toward the center of the cell in the colcemid-treated samples.

Using scanning electron microscopy, we looked at cells that were in all portions of the cell cycle: interphase cells were flat and tightly adhered to the coverslip, whereas mitotic cells were rounded and loosely attached. In this way, alterations of the surface that appear in mitosis or after the cells have passed into interphase could be observed. Figure 4 shows the surface of V79 control cells, which were incubated at 37°C before preparation for microscopy. The cells show numerous microvilli and a small number of surface blebs. Also shown are two rounded cells, which appear to have been recently formed from a cell division. The colcemid-treated cells, which were held at 37°C, appear similar to the untreated cells.

Cells incubated at 45°C for 30 min remained well attached to the coverslip and were similar in appearance to those treated at 37°C, except for fewer microvilli on the cell surface (Figure 5). The number of blebs on the cell surface was not increased by heating under these conditions.

Discussion

There have been other reports that support our findings. Other mitotic poisons increase resistance to radiation damage in cultured cells. Colchicine has been reported to protect plant cells from X-ray-induced chromosomal aberrations by an unknown mechanism (Mandal and Basu, 1983). Iliakis and Nusse (1984) have also shown that nocodazole protected G1, S and G2-irradiated Ehrlich ascites cells, which were able to repair potentially lethal damage during the time in arrested mitosis. A similar process can be envisioned for the protective effect of colcemid on thermal damage.

Szmigielski et al. (1977) have reported the contrary result, i.e., colcemid addition enhances the effect of hyperthermia and Hofer and Mivechi (1981) have reported that colcemid addition produces no effect in murine sarcoma cells. However, they did not take the cell cycle effects into account; hence, they may have been measuring the effects of an increased number of cells with heightened thermal sensitivity in mitosis.

The fate of the cytoskeleton after heating in G1 phase has been reported to play a role in cell survival. Wachsberger and Coss (1990) found a positive correlation between recovery from heat-induced cytoskeletal disruption and survival. This contrasts with our results using mitotic cells where colcemid-induced disruption of the microtubules and microfilaments before and during heating appears to enhance survival. Perhaps the colcemid-induced breakdown of microtubular elements and banding of intermediate filaments into cables in mitotic cells (Szekely et al., 1983; Blose and Chacko, 1976; Forry-Schaudies et al., 1986) reduces the damage produced when the microfilaments are subsequently heated. Collier and Schlesinger (1986) have suggested that heat shock proteins aid in the reformation of the intermediate filament network after stress. The stress of colcemid addition may prime the formation of heat shock proteins, which react to the heat stress. Thus, the position in the cell cycle is an important determinant of the interaction of cytoskeleton structure and heat.

The electron microscopy pictures show structural alterations in the mitochondria and the chromatin caused by the heating. The colcemid-treated and untreated samples were similar except for the aggregation of the mitochondria and organelles inside the endoplasmic reticulum in the colcemid-treated samples. The blebbing reported by Borrelli et al. (1986) in G1 phase CHO cells was not observed in the mitotic V79 cells with or without colcemid. Mitochondria were altered in V79 cells by the heat shock. We observed damage similar to that reported by Welch and Suhan (1985) and Arancia et al. (1989). The cristea were separated with dark staining material in the mitochondrial space and dense particles were seen. The chromatin was also more diffuse in the heated samples regardless of whether or not colcemid was present. Thus, although the heating produced a number of alterations in the ultrastructure of the cell, the changes were not negated by colcemid addition.

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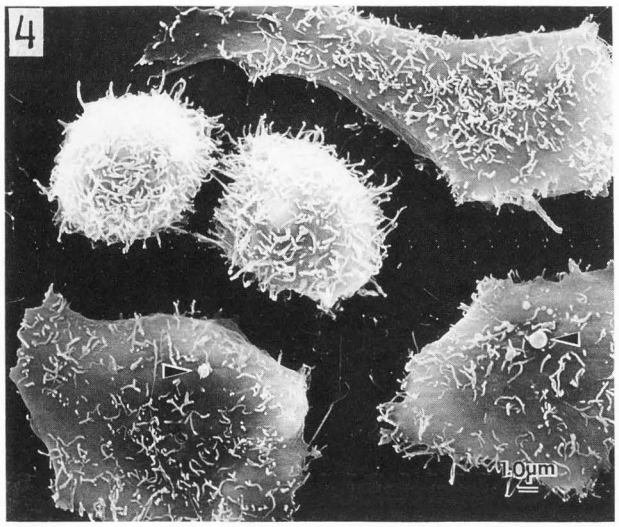

Fig. 4. A scanning electron micrograph of cells incubated at 37°C without colcemid. Surface blebs are shown with arrows.

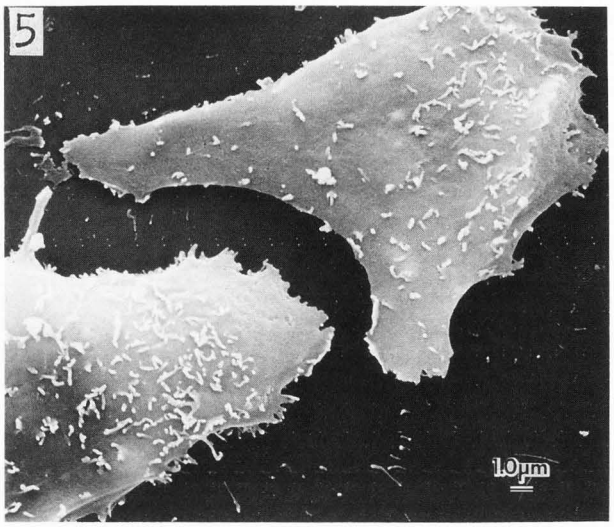

Fig. 5. A scanning electron micrograph of cells incubated for 30 min at 45°C without colcemid.

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Discussion with Reviewers

Z. Somosy: Is there any data on the effect of synchronization of cells with colcemid and heat effects?

Authors: Mitotic selection using colcemid and other methods have been used to select cells for synchronization experiments. Regardless of the synchronization method, however, the same pattern of sensitivity to heat has been reported: M and late S phase cells are the most heat sensitive (Bhyuyan BK, Day KJ, Edgerton CE, Ogunbase O. (1977). Sensitivity of different cell lines and of different phases in the cell cycle to hyperthermia. Cancer Res. 37, 3780-3784; Raaphorst GP, Azzam EI. (1984). A comparative study of heat and/or radiation sensitivity of V79 cells synchronized by three different methods. Proc. Fourth Int. Symp. Hyperthermic Oncology, 1, 301-302; Westra A, Dewey WC. (1971). Variations in sensitivity to heat shock during the cell-cycle of Chinese hamster cells in vitro. Int. J. Radiat. Biol. 19, 467-477). To our knowledge, the work of Szmigielski et al. (1977) is the only report with cells heated in the presence of colcemid. They report a decrease in viability; however, they used unsynchronized cells rather than the mitotic cells we have studied.

R.A. Coss: I would highly recommend that the authors generate complete survival curves in the presence and absence of colcemid. By doing this the PE problem would be circumvented, and colcemid protection would be easily demonstrated.

L. Yasui: My major concern is the interpretation of the survival data and the lack of full survival curves.

Authors: The data summarized in Tables 1 and 2 are the results of experiments with six repeats at 42°C and seven repeats at 45°C. In all cases, survival in the presence of colcemid was higher, and the relative plating efficiency at the elevated temperature compared to that at 37°C was greater than one. Additional flow cytometry experiments, which we have carried out (not reported here), further suggest that both the colcemid-treated and non-treated cells are held in mitosis for an hour after heating to 45°C; thus, the differences reported are not simply a result of mitotic and G1 cells being plated after heating in the two situations. In view of this, we are confident that the observed effects are real. We agree with the reviewers that complete survival curves would strengthen the conclusions and eliminate any doubts raised by the variable plating efficiency; however, institutional changes have made it impossible for us to add to the work reported in the text.